Brain Plasticity in the Developing Brain

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Abstract

The developing normal brain shows a remarkable capacity for plastic change in response to a wide range of experiences including sensory and motor experience, psychoactive drugs, parent–child relationships, peer relationships, stress, gonadal hormones, intestinal flora, diet, and injury. The effects of injury vary with the precise age-at-injury, with the general result being that injury during cell migration and neuronal maturation has a poor functional outcome, whereas similar injury during synaptogenesis has a far better outcome. A variety of factors influence functional outcome including the nature of the behavior in question and the age at behavioral assessment as well as pre- and postinjury experiences. Here, we review the phases of brain development, how factors influence brain, and behavioral development in both the normal and perturbed brain, and propose mechanisms that may underlie these effects.

Keywords

brain development, prefrontal cortex, recovery of function, types of plasticity

1 INTRODUCTION

The development of the brain and behavior is guided not only by a basic genetic blueprint but also by a wide range of experiences that shape the emerging brain. Brains exposed to different environmental events such as sensory stimuli, stress, injury, diet, drugs, and social relationships show a unique developmental trajectory. The explosion of epigenetic studies in the past few years has also demonstrated that prenatal, and even preconceptual, experiences modify the organization of neural networks. The goal of this review is to consider the manner in which the developing brain can be modified by a range of prenatal and postnatal factors that can influence how the brain responds to other experiences later in life. Our focus will be on the
neocortex of the rat because the majority of our knowledge regarding the modulation of brain development is based on studies of neocortical development. We begin with a brief review of the stages of brain development followed by a consideration of how factors influence brain development and behavior.

2 STAGES OF BRAIN DEVELOPMENT

The Roman philosopher Seneca concluded that an embryo is an adult in miniature and the purpose of development was to grow bigger. By the twentieth century, it became clear that this was not the case. Today, development can broadly be divided into two phases. In mammals, the first phase is in utero and reflects a genetically determined sequence of events that can be modulated by the maternal environment. The principal developmental stages here are neural generation and migration. The second phase, which is largely postnatal in species such as the rat, but both pre- and postnatal in species such as humans where brain development is more prolonged. The second phase of development is a period in which the emerging connectivity of the brain is very sensitive to both environmental stimuli but also to the patterns of brain activity produced by previous experiences.

Table 1 summarizes seven general stages of brain development characteristic of all mammals. The generation of neurons in rats begins on embryonic (E) day 10.5–11 when the neural tube is formed with the generative zone, called the subventricular zone, as the primary “nursery” (for reviews, see Bayer and Altman, 1991; Semple et al., 2013). The cerebral cortex is mainly generating cells from E15–21. Once generated in the subventricular zone, the putative neurons migrate to their appropriate locations in the developing cortical plate. The migratory process can take 2–5 days, depending upon the final location (see Fig. 1) (Bayer and Altman, 1991; Hicks and D’Amato, 1968). There are two types of migration. Pyramidal cells migrate from the ventricular zone along radial glia to their respective cortical layers (Rakic, 1972). Interneurons take a tangential trajectory in what Bayer and Altman call the lateral migratory stream as illustrated in Fig. 1. These cells are generated more laterally in the ventricular zone in a region known as the ganglionic eminence (Cuzon et al., 2006; Jimenz et al., 2002). Errors in the migration process, including abnormal cell proliferation, abnormal timing or migration, or abnormal cortical

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organization, have been implicated in disorders such as epilepsy, autism, and schizophrenia among others (e.g., Mochida and Walsh, 2004). Furthermore, prenatal exposure to drugs, such as diazepam (a GABA agonist), can alter migration patterns (Cuzon et al., 2006).

Once the cells reach their appropriate locations, there is a rapid differentiation into cell types and growth of dendrites and axons, a process that peaks at 7–10 days postnatal (P). Synapse production begins once neurons mature with a rapid increase beginning around P10. Micheva and Ceaulieu (1996) counted the number of GABA and non-GABA cells and found that the cell volume peaks at about P30 in

![Diagram of cell migration](image.png)

**FIGURE 1**
A summary figure showing cell migration in the anterior and middle parts of the developing neocortex. Neurons generated in the ventricular zone (striped layer) migrate radially to the dorsal cortical plate in 2 days, migrate laterally to the lateral cortical plate in 3 days and to the ventrolateral cortical plate in 4 days. Some cells generated in the ventricular zone migrate in the lateral cortical stream for up to 4 days and accumulate in the reservoir. Some migrate into the pyriform cortex, whereas others migrate to as yet unidentified areas in the basal telencephalon.

*From Bayer and Altman (1991), with permission.*
somatosensory cortex. There was no further change in synapse number at P60, which was the oldest age that the authors examined. It is well documented that in primates there is an overproduction and later elimination of synapses (e.g., Huttenlocher, 1984; Petanjek et al., 2011), which in humans continues well into the third decade of life. However, the possible overproduction and pruning of synapses is not well studied in the rat. Although Micheva and Beaulieu did not see any change up to P60 in somatosensory cortex, it is likely that at least some regions are shedding synapses after P60. Van Eden et al. (1990) showed a decline in cortical thickness in prefrontal cortex from P60 to P90 and Vinish et al. (2013) showed a decrease in spine density over a similar time period in medial prefrontal cortex (mPFC). These results imply that a more systematic analysis of synaptic formation beyond weaning is required in the rat. The need for neuronal and synaptic pruning is likely related to the uncertainty in the number of neurons that will reach their appropriate destinations and the appropriateness of the connections that they form.

Three features of brain development are especially important in the current context. First, the cells lining the subventricular zone include stem cells that remain active throughout life. These stem cells can produce neural or glial progenitor cells that are able to migrate into the cerebral white or gray matter in adulthood. The role of these cells is poorly understood as they appear to remain quiescent for extended periods but can be activated to produce neurons or glia, especially after injury (e.g., Kolb et al., 2007). Second, cells in the dentate gyrus of the hippocampus are generated there throughout life, although this production declines with aging. These cells appear to play a role in functions such as memory (e.g., Spanswick and Suthe

3 GENERAL TYPES OF BRAIN PLASTICITY

Changes in the brain can be shown at many levels of analysis (see Table 2) ranging from behavior to molecules. There is no correct level of analysis, but rather the measure of plasticity must be suited to the research question being asked. Noninvasive

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<td>Behavior</td>
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imaging is appropriate to study experience-dependent changes in humans but is far more difficult to use in laboratory animals. An advantage of using laboratory animals, however, is that it is possible to measure anatomical and molecular changes in postmortem tissue of animals with different experiences. Our bias in the current review is to emphasize the correlation between synaptic change, using Golgi-type stains, and epigenetic analyses, looking for changes in gene methylation and/or gene expression.

Three types of plasticity can be distinguished in the normal brain: experience-independent, experience-expectant, and experience-dependent (Greenough et al., 1987; Shatz, 1992). Experience-independent plasticity is largely a prenatal developmental process. It is impractical for the genome to specify the connectivity of every connection in neuron development. Instead, the brain produces a rough structure in which there is an overproduction of neurons, and later, connections, that are sculpted in response to internal and external events. A good example of experience-independent plasticity is the development of the eye-specific layers of the lateral geniculate nucleus (LGN) of the cat (Campbell and Shatz, 1992). Axons arriving from the retina eventually terminate in separate layers in the LGN, but retinal cells initially also send axonal branches to the layer for the other eye. In order to segregate the layers correctly, the retinal ganglion cells spontaneously fire so as to correlate their firing with nearby cells but independent of those in the other eye. Cells that fire together increase their connections, whereas those out of synch weaken their connections and eventually die out. This type of plasticity, which is independent of external sensory input, allows the nervous system more precise in connectivity without requiring overwhelmingly complex genetic instructions.

Experience-expectant plasticity largely occurs during development. A good example is the development of ocular dominance columns found in the primary visual cortex. These alternating columns provide a mechanism for the inputs from the right and left eyes to be combined to produce binocular vision. Wiesel and Hubel (1963) showed in kittens that if one eye is kept closed after birth, the open eye expands its territory, leading to shrinkage of the column related to the closed eye. When the closed eye is eventually opened, its vision is compromised.

Finally, experience-dependent plasticity refers to a process of changing neuronal ensembles that are already present. This can be seen in situations such as when animals learn problems (e.g., Greenough and Chang, 1989), when topographic maps expand or shrink in response to experience (e.g., Blake et al., 2002), when animals receive intense environmental manipulations (e.g., Greenough and Chang, 1989), injury (e.g., Kolb, 1995), or in response to abnormal experiences such as psychoactive drugs (e.g., Robinson and Kolb, 2004). These types of experiences both increase and decrease synapse numbers, often in the same animals, but in different brain regions (see below). The key point is that the synaptic changes reflect modifications of a basic phenotype formed in development. It is important to note that although it is often assumed that experience-dependent plasticity largely reflects the addition of synapses, it may be seen both in the addition and/or pruning of synapses.
4 FACTORS INFLUENCING BRAIN DEVELOPMENT IN THE NORMAL BRAIN

When researchers began to study experience-dependent changes in the brain, there was a belief that changes would be most dramatic in severely impoverished conditions, such as being raised in the dark (e.g., Reisen, 1961). Such extreme experiences did profoundly alter brain development, but it soon became clear that a wide range of experiences, and even fairly innocuous-looking experiences, could also produce large changes in the brain (see Table 3).

4.1 Complex housing

The simplest and most dramatic way to manipulate experience is to compare the brain structure in animals placed in complex environments (sometimes called enriched environments) versus that of animals housed in standard laboratory caging. The original studies by the group at Berkley (e.g., Rozenweig et al., 1967) found changes in cortical thickness and neurochemistry; however, it is now known that there are changes in brain size, cortical thickness, neuron size, dendritic branching, spine density, synapses per neuron, glial numbers and complexity, expression of neurotransmitters and growth factors, and vascular arborization (e.g., Greenough and Chang, 1989; Sirevaag and Greenough, 1988). Such changes are correlated with a wide range of enhanced cognitive and motor abilities.

It has only been more recently that the effect of complex housing on brain development has been investigated, especially examining the effects on the visual system (see review by Baroncelli et al., 2010). Complex housing from birth accelerates the maturation of visual acuity, which is associated with electrophysiological changes.
Interestingly, complex housing can promote the development of the visual system even in the absence of visual stimulation in animals housed in the dark (Bartoletti et al., 2004). In fact, the latter study showed that the nonvisual effects of complex housing could reverse the effects of raising animals in the dark. Although the mechanism of this effect is not known, one possibility is that pups raised in complex environments receive more maternal care (Sale et al., 2004), which is known to be a strong factor in changing brain development (see below).

There are few studies of other cortical regions but one particular study showed enhanced auditory functioning in rats raised in complex environments (Cai et al., 2009). Early complex housing also alters the development of the parietal cortex. Kolb et al. (2003a) placed weanling rats in complex environments and compared the cortical changes to animals placed in the same environments as adults. Whereas adult rats showed increased dendritic length and spine density after 90 days, juvenile rats showed a similar increase in dendritic length but a decrease in spine density. That is, the young animals showed a qualitatively different change in the distribution of synapses on pyramidal neurons compared to the older animals. This result was surprising, so the researchers wondered what earlier experience might do. In a follow-up experiment, pregnant dams were placed in complex environments for 8 h a day beginning a week prior to their pregnancy and then throughout the 3-week gestation. The brains of their offspring were examined in adulthood and showed a decrease in dendritic length and an increase in spine density in parietal cortex.

![Figure 2](image-url)

**FIGURE 2**

Average global DNA methylation levels in the hippocampus and frontal cortex of offspring of males who were housed in complex environments for 28 days prior to mating with a control female and females who were housed in complex environments for 7 days prior to conception and for the duration of the pregnancy. 

*After Mychasiuk et al. (2012).*
Thus, complex housing has qualitatively different effects at different developmental ages. In a parallel study, Pena et al. (2009) found enduring effects of complex housing from weaning until adulthood on pituitary–adrenal function, social behavior, and cognitive behavior in adulthood.

Early complex housing has also been shown to attenuate the effects of exposure to both methylphenidate and amphetamine later in life. Alvers et al. (2012) found a reduction in the self-administration of low, but not high, doses of methylphenidate, whereas Li, Robinson, and Kolb (unpublished observations) found that lifetime complex housing reduced amphetamine-induced behavioral sensitization as well as the dendritic changes in mPFC and nucleus accumbens.

Finally, Mychasiuk et al. (2012b) placed male rats in complex environments for 28 days before mating the males with control females and compared the epigenetic effects to maternal housing as in the earlier Gibb (2004) study (Fig. 2). The offspring of the complex-environment housed males showed a significant decrease in gene methylation, reflecting the increased expression of about 1000 genes. More surprising, however, was that the levels of gene expression changes were remarkably similar to those observed in the offspring of females who were housed in similar complex environments while pregnant.

In sum, complex housing during development has profound and enduring effects on brain development and function. A key question relates to exactly what it is about the complex housing experience that is altering brain development.

**FIGURE 3**
The effects of neonatal tactile stimulation on spine density in mPFC (Cg3), OFC (AID), and amygdala. Similar results were shown for dendritic length and branching.

After Richards et al. (2012).
4.2 Sensory and motor experience

As noted earlier, one explanation for the complex rearing effects is that there was an increase in maternal behavior, including licking and grooming of the infants. Schanberg and Field (1987) showed that tactile stimulation of preterm infants accelerated growth and led to earlier release from hospital. More recently, it has been shown that tactile stimulation in preterm infants accelerates EEG maturation and visual functions, as well as increasing serum levels of insulin growth factor I (IGF-I) and growth hormone paralleling results found in rats (Field et al., 2008; Guzzetta et al., 2009). Further studies in rats have also shown that early tactile stimulation improves motor and cognitive functions in adulthood as well as increasing dendritic length and spine density in mPFC (Fig. 3) (Richards et al., 2012) and the expression of fibroblast growth factor-2 (FGF-2) in skin and brain (Gibb, 2004). Early tactile stimulation (either stimulation of the pregnant dam or postnatal stimulation of the pups) attenuates the behavioral and anatomical effects of amphetamine in adulthood (Muhammad and Kolb, 2011a,b; Muhammad et al., 2011). And, as discussed below, tactile stimulation dramatically improves recovery from early cortical injury (Kolb and Gibb, 2010). There is little doubt that tactile stimulation has an effect on cortical development that is nearly as large, although somewhat different from, complex housing.

4.3 Psychoactive drugs

Alcohol has long been associated with compromised brain development, but only recently it has been shown that many other psychoactive drugs, including prescription drugs, alter brain development. Exposure to psychoactive drugs in adulthood produces persistent structural changes to cells in both mPFC and orbital prefrontal cortex (OFC) and nucleus accumbens (Robinson and Kolb, 2004). There is now growing evidence that the prenatal administration of a wide range of psychoactive drugs including nicotine, diazepam, and fluoxetine chronically alters both neuronal structure and cognitive and motor behaviors (e.g., Kolb et al., 2008; Muhammad et al., 2013; Mychasiuk et al., 2013a,b). Similarly, administration of amphetamine, methylphenidate, haloperidol, and olanzapine in the juvenile period also leads to impaired behavior and dendritic aberrations in rats examined in adulthood (Diaz Heijtz et al., 2003; Frost et al., 2010; Milstein et al., 2013; Vinish et al., 2013).

One key question is whether early exposure to psychoactive drugs alters brain plasticity later in life. If adult rats are given nicotine, cocaine, or amphetamine and later placed in complex environments, neuronal plasticity is blocked (Hamilton and Kolb, 2005; Kolb et al., 2003b). If rats are given nicotine prenatally and placed in complex environments in adolescence, there is a complex array of dendritic/spine changes including a partial reversal of the effects of nicotine as well as a blockade of the effects of the complex housing (Muhammad et al., 2013).
4.4 Parent–child relationships

Developing mammals are dependent upon their parents and parent–child relationships are critical for brain development. Variations in the pattern of early parent–infant interactions can initiate long-term developmental effects that persist into adulthood (Myers et al., 1989). This has been most extensively studied in mother–infant interactions in rodents where the time spent in contact and the amount of maternal licking and grooming of the infants correlate with a variety of somatic and behavioral outcomes. For example, Meaney and his colleagues have shown that maternal–infant interactions modulate a variety of emotional and cognitive behaviors in adulthood, in part through modifications of the hypothalamic–adrenal stress response (e.g., Cameron et al., 2005) as well as changes in gene expression in hippocampus (Weaver et al., 2005). Other studies have shown changes related to maternal–infant interactions in the hypothalamus and amygdala (Fenoglio et al., 2006; Moriyama et al., 2013), as well as mPFC and OFC (Muhammad and Kolb, 2011a,b).

4.5 Peer relationships

Peer relationships, and especially play, have been known to influence the development since the studies of Harlow (e.g., Harlow and Harlow, 1965). The prefrontal cortex plays a central role in play behavior and, in turn, its development is strongly influenced by play. Perinatal injury to the mPFC or OFC regions compromises play behavior, although in different ways (e.g., Pellis et al., 2006). Similarly, the amount of play that young rats are allowed to engage in alters the development of prefrontal cortex. Neurons of the mPFC region respond to the amount of play but not the number of playmates, whereas the OFC responds to the number of playmates and not the amount of play (Bell et al., 2010). Furthermore, early experiences including prenatal stress and tactile stimulation alter play behavior and prefrontal cortex (e.g., Muhammad et al., 2011). Indeed, it seems likely that any treatment that alters play behavior will alter prefrontal development and function. For example, the manipulation of juvenile play behavior also changes the brain’s response to psychomotor stimulants (Himmler et al., 2013a,b).

4.6 Stress

Although it has long been known that stress alters the brain and behavior of adults, it is only recently that the role of perinatal stress has been appreciated. For example, prenatal stress is now known to be a risk factor in the development of schizophrenia, attention-deficit hyperactivity disorder (ADHD), depression, and drug addiction (Anda et al., 2006; van den Bergh and Marcoen, 2004). Studies with laboratory animals have also shown that perinatal stress produces a wide range of behavioral abnormalities, including an elevated and prolonged stress response, impaired learning
FIGURE 4
Mean (± SEM) total number of branch bifurcations (dendritic branching), number of excitatory synapses, and spine density in Nucleus accumbens (NAc), OFC (AID), and mPFC (Cg3Basilar field; Cg3Apical field). The mean branch order and number of synapses are in the same scale shown on the right vertical axis for NAc, AID, and Cg3B but are in a different scale for the Cg3A, which is on the left vertical axis. The letters “a” and “b” represent the comparisons of the effects prenatal stress (PS) and maternal separation (MS), respectively, compared to controls. The letter “c” represents comparisons between PS and MS (ps <0.05 or better).

After Muhammad et al. (2012).
and memory, altered social and play behavior, increased anxiety, deficits in attention, and increased preference for alcohol (Weinstock, 2008).

Stress has long been known to alter the prefrontal cortex of adults (e.g., Liston et al., 2006), but it has become apparent that the changes in the developing prefrontal cortex are very different. For example, adult stress leads to a decrease in spine density in mPFC, but an increase in orbital cortex (Liston et al., 2006). In contrast, Murmu et al. (2006) found that prenatal stress in degus from E16 to E21 produced a decrease in both spine density and dendritic length in both mPFC and OFC in adulthood. Using a somewhat different paradigm, our laboratory exposed rats to gestational stress at E12–16 and found an increase in spine density in both mPFC and OFC when the brains were examined at weaning or in adulthood (Muhammad et al., 2012; Mychasiuk et al., 2012). We also compared the effects of prenatal stress and postnatal maternal separation and found very different effects of the two stressors (see Fig. 4). Thus, the effects of perinatal stress vary with the nature of the stress, the precise embryonic age of the stress, and the age at which the brain is examined.

One explanation for the difference between the Murmu results and our results may be related to differences in epigenetic changes related to intensity of the stress. Mychasiuk et al. (2012) found that mild gestational stress increased global methylation in prefrontal cortex (using a combined sample of mPFC and OFC), whereas greater stress had the opposite effect. A whole-genome microarray showed that over 700 genes in the prefrontal cortex and hippocampus were differentially expressed following prenatal stress, with most genes being downregulated.

The effects of gestational stress can be surprisingly subtle. Mychasiuk et al. (2011a,b,c,d) housed pregnant females together for the duration of their pregnancies. One rat received stress at E12–16 while the other (the bystander) did not. The offspring of both dams showed significant changes in methylation, gene expression, and dendritic organization but the patterns of gene expression and dendritic changes were qualitatively different (Mychasiuk et al., 2011a,b,c,d). It appears that both dams were stressed but in different ways, which led to differential effects on the offspring brains. One obvious question is to ask how prenatal stress affects the brain response to other postnatal developmental experiences such as complex housing, tactile stimulation, play, and so on.

4.7 Gonadal hormones

During development the most obvious effect of exposure to gonadal hormones is the prenatal differentiation of the genitals. But the same gonadal hormone receptors are found in the brain, so it would be surprising if there were not sex differences there too. There are clear differences in the adult human brain (e.g., Goldstein et al., 2001) and MRI studies of human children have shown large differences in the rate of brain development in the two sexes (O’Hare and Sowell, 2008), with the female brain reaching its adult volume 2–4 years earlier than the male brain. Studies in rats have shown that neurons in the mPFC have larger dendritic fields and higher spine density
in males than females, whereas the opposite is found in OFC (e.g., Kolb and Stewart, 1991). Furthermore, there are sex differences in the effects of many perinatal experiences including gestational stress, complex housing, and injury (e.g., Kolb and Stewart, 1995; Mychasiuk et al., 2011a,b,c,d).

### 4.8 Intestinal flora

Gut microbiota have adapted to a symbiotic relationship with many animals. Soon after birth, the gut of mammals is populated by a variety of indigenous microbes that influence both gut and liver functions (e.g., Bjorkholm et al., 2009; Hooper and Gordon, 2001). There are many similarities in the neurochemical organization of the enteric and central nervous systems, so it is reasonable to speculate that gut microbiota might influence brain function. Indeed, epidemiological studies have shown an association between neurodevelopmental disorders including autism and schizophrenia and microbial infections early in life (e.g., Finegold et al., 2002; Mittal et al., 2008). Diaz Heijtz et al. (2011) manipulated gut bacteria in newborn mice and found that gut bacteria influence motor and anxiety-like behaviors, which were associated with changes in the production of synaptic-related proteins in cortex and striatum. This finding is important because it provides a mechanism whereby infections during development could influence brain development as well as a reason why results in different laboratories could be different depending upon dietary selection and which gut microbiota are present in the respective colonies.

### 4.9 Diet

There is an extensive literature on the effects of caloric- and/or protein-restricted diets on brain and behavioral development (e.g., Lewis, 1990) but little research on brain plasticity and restricted diets per se. Similarly, little is known about the effects of enhanced diets on brain development. It is reasonable to predict that brain development might be facilitated by vitamin and/or mineral supplements. Dietary choline supplementation during the perinatal period leads to enhanced spatial memory in various spatial navigation tasks (e.g., Meck and Williams, 2003; Tees and Mohammadi, 1999) and increases the levels of nerve growth factor in hippocampus and neocortex (e.g., Sandstrom et al., 2002). Halliwell and Kolb (2003) did similar studies and found increased dendritic length in pyramidal cells across the cerebral cortex and CA1 of the hippocampus with choline supplementation.

Halliwell (2011) also studied the effects of a vitamin/mineral supplement to the food of lactating rats. The same dietary supplement was reported to improve mood and aggression in adults and adolescents with various disorders (Leung et al., 2011) and reduced social withdrawal and anger in children with autism (Mehl-Madrona et al., 2010). Analysis of the adult offspring of lactating rats fed the same supplement found an increase in dendritic length in pyramidal neurons in mPFC and parietal
cortex but not in OFC. Furthermore, the same diet facilitated recovery from perinatal mPFC lesions in rats.

5 BRAIN DEVELOPMENT AFTER EARLY BRAIN INJURY

The first systematic studies on the effect of brain injury in development were done by Margaret Kennard, beginning in the 1930s (e.g., Kennard, 1942). She made unilateral motor cortex lesions in infant and adult monkeys and found milder impairments in her young animals. This led her to suggest that there was some change in the cortical organization of the infants that supported more normal behavior (Kennard, 1942). Although Kennard was aware that her monkeys still had deficits, she is credited with the general idea that “earlier is better,” a conclusion that Teuber (1975) referred to as the Kennard Principle. Hebb (1945, 1949) reached a different conclusion, however. He was studying the effects of early frontal lobe injury in children and concluded that these children had worse outcomes than adults with similar injuries. He suggested that the early frontal injury was interfering with the normal development of neural networks needed to support many adult behaviors (see also Stiles, 2012). The conclusions of Kennard and Hebb would appear to be at odds, but over the past 30 years’ extensive studies of monkeys, cats, and rats with cortical injuries have shown that both views are partially correct. The outcomes depend upon the precise age at injury, the behavioral measurements used, the age at assessment, and whether the injury is unilateral or bilateral.

5.1 Age at injury

Age at injury refers not to the actual postnatal age of animals but to their developmental age. Rodents and carnivores are born less mature than primates, so birth date is not helpful in comparing across species. We will consider the effects of cortical lesions in rats first, with an emphasis on frontal lesions because as in monkeys and cats, this is where most of the studies are focused. Although it is common in the perinatal ischemia literature to make lesions on postnatal day 7 (P7) with rats to mimic birth asphyxia in humans, this age misses much of the story.

It has become clear that in rats and mice, damage on days 1–5 has devastating consequences on behavior, with the effect generally being worse the earlier the postnatal injury, regardless of which cortical region is damaged. In contrast, similar damage on days 7–12 permits much better functional outcome, and depending upon the behavioral measure, there can be surprisingly normal behavior, despite the fact that the brain is significantly smaller than normal (see Table 2). There is still a better outcome than observed in adults when mPFC lesions are incurred in early adolescence. We are aware of only one study of prenatal cortical lesions in rats and there was remarkably normal behavior, in spite of a highly abnormal brain (Kolb et al., 1994a,b).
There were, however, a series of studies by Sam Hicks in the 1950s in which ionizing radiation was used to alter brain development, which in some cases led to surprisingly good outcomes in spite of abnormal brains, provided there was no hydrocephalus (e.g., Hicks and D’Amato, 1961).

In sum, functional outcome is good if injury is during the latter part of neurogenesis (E18) but poor if it is during migration and the beginning of synaptogenesis. It is better again after migration is done and synaptogenesis is burgeoning. We must note that although we have focused on mPFC lesions, a similar pattern is seen after OFC, motor cortex, posterior cingulate cortex, posterior parietal cortex, visual cortex, and auditory cortex lesions (see review by Kolb et al., 2010). As might be expected, however, recovery is not equivalent across all regions, with posterior injury allowing less recovery than anterior lesions.

Results from studies of cats and monkeys present a similar pattern, although the dates vary because of differences in gestational rate. Villablanca and his colleagues conducted an extensive series of studies on the behavior of cats with frontal or prefrontal injuries (e.g., Villablanca et al., 1993). Cats are an interesting comparison to the rat and monkey because they are embryologically older than rats at birth with a gestation period of about 65 days, but they are much younger at birth than monkeys. Overall, Villablanca has found that although cats with prefrontal lesions shortly after

FIGURE 5
Photographs of brains of animals given midline frontal cortex lesions on postnatal day 10 and then sacrificed on postoperative days 1, 3, 8, or 23. The lesion cavity is evident at day 1 but by day 23 is only visible as a scar. By day 90 (not shown) the scar is no longer visible.

After Kolb et al. (1998a,b).
birth show good recovery relative to animals with lesions later in life, cats with prenatal lesions have severe behavioral impairments. Thus, the newborn cats appear similar to P10 rats, whereas the prenatal cats are similar to P1–6 rats.

Monkeys are different again. They are born much older than rats, cats, or even humans. Although Kennard reported better outcomes with infant lesions, as did Harlow et al. (1964), the bulk of the later evidence largely by Goldman and colleagues did not report this (see reviews by Goldman, 1974; Goldman et al., 1983). In contrast, however, prenatal lesions in monkeys allow substantial recovery (Goldman and Galkin, 1978). The prenatal lesions are more similar in embryological time to newborn cats and P10 rats. We can predict that if Goldman and Galkin had made their prenatal lesions even earlier, the outcome would be similar to the prenatal lesions in cats and lesions in newborn rats.

But what are the anatomical correlates of this recovery? Studies of unilateral motor cortex lesions (e.g., Hicks and D’Amato, 1975) found anomalous projections from the intact hemisphere to the spinal cord, leading to the presumption that these projections supported the functional recovery. They likely did. Similarly, there are anomalous projections in the cat visual system that are associated with functional recovery (Payne and Lomber, 2001) as well as extensive anomalous connections after neonatal hemidecortication in both rats and cats that are correlated with recovery. But anomalous projections are also associated with poor outcomes. For example, whereas rats with P1 mPFC lesions have significant abnormal connections, P10 animals do not (Kolb et al., 1994a) but it is the P10 animals that show recovery, not the rats with the rewired brain. There are several other types of anatomical changes that support recovery in P10 rats. For example, there is widespread dendritic hypertrophy of cortical pyramidal neurons as well as increased spine density. Furthermore, there is evidence that for at least some types of P10 lesions, namely mPFC and posterior cingulate, there is spontaneous neurogenesis that fills the lesion cavity (Fig. 5) (Kolb et al., 1998a,b). The neurons that reform the frontal region establish connections with subcortical regions such as the striatum and have electrical activity that is nearly normal (Driscoll et al., 2007). In the process of studying the apparent regeneration of the lost cortex, we discovered serendipitously that injections of the mitotic marker, bromodeoxyuridine (BrdU), on E12–14 disrupt the postnatal activity of stem cells (Kolb et al., 1999) and completely block the postinjury neurogenesis (Kolb et al., 1998a,b, 2012). Not surprisingly there is no functional recovery if there is no neurogenesis.

Finally, one other mechanism appears to be the survival of neurons in the thalamus that should have degenerated after the injuries. Normally, if cortical regions are damaged, the thalamocortical projection cells die. This is true after neonatal visual cortex lesions but not after prefrontal lesions. Cells in the dorsal medial thalamus, which projects to prefrontal cortex, survive and form connections with remaining frontal regions in both monkeys and rats (Goldman and Galkin, 1978) and rats (Kolb and Nonneman, 1978), although this likely depends on the age at injury. Van Eden et al. (1998) used unbiased stereology to count neurons after P6 mPFC lesions in newborn rats (Goldman and Galkin, 1978).
lesions and found no difference from adults with similar lesions. This study should be repeated with P10 lesions.

5.2 Behavior specificity
As a rule of thumb, cognitive functions show better functional recovery than motor functions, which in turn show better recovery than species-typical behaviors, which show virtually no recovery regardless of the age at injury (e.g., Kolb and Whishaw, 1981). It appears that it is easier for the brain to form new neuronal ensembles to solve cognitive tasks than motor tasks and the neural circuits underlying species-typical behaviors are relatively hard-wired and not easily replaced.

One surprising effect of early lesions is seen in spatial behaviors. Rats with P1–5 show poor recovery, which is not surprising given that adult rats with similar lesions are also impaired. What is unexpected, however, is that lesions elsewhere in the cerebral cortex at P1–5 also produce deficits in spatial behaviors even though similar adult lesions do not. This effect is not unique to rats, however, as children with early cortical lesions in either hemisphere show the same result (e.g., Akshoomoff et al., 2002). This is in marked contrast to the recovery of language in children with perinatal injuries to the speech areas, although the recovery of language is less complete than was originally believed (Thai et al., 1991).

5.3 Age at assessment
One of challenges in assessing the effects of early brain injury is the problem of knowing when to investigate the behavior. This is nicely illustrated by the early studies of Goldman (e.g., Goldman, 1974). She was initially impressed with apparent recovery of functions after frontal lobe injuries in infant monkeys but as she continued her studies it became clear that she, and others before her, had overestimated the extent of recovery because the animals were tested when they were still young. Thus, she found that animals with dorsolateral prefrontal lesions became progressively more impaired at cognitive tasks such as delayed alternation as the brain developed (Goldman et al., 1983).

She tested the idea that age was important directly by studying the behavioral effect of reversibly cooling the otherwise normal prefrontal cortex of monkeys at different ages. On a task that monkeys with adult lesions are impaired at (delayed response), she found that when she cooled the cortex at 9–16 months of age the animals performed as well as they did in the uncooled state. However, when tested at 19–31 months the animals were impaired, and this impairment grew progressively larger as they grew older. Thus, she was able to show that animals with early cortical lesions “grow into their deficits,” an observation also made in children with congenital brain injuries (Banich et al., 1990) and hamsters with mPFC lesions on P2 (Kolb and Whishaw, 1985).
But age at assessment can work in reverse as well. When rats were given mPFC lesions on P1 or P10 and then behaviorally tested at P22–25, both groups were severely impaired relative to controls (Kolb and Gibb, 1993). However, when the rats were tested at P52–55, the P10 rats were no longer impaired whereas the P1 rats were. The recovery of the P10 rats was correlated with hypertrophy of cortical pyramidal neurons that was not present in the brains of P25 animals. Thus, not only can animals grow into deficits but out of them too.

**FIGURE 6**

Performance on a skilled reaching task by rats with adult or P5 unilateral lesions of varying sizes (small, medium, and large) of the motor cortex or the entire neocortex (hemidecorticate). Reaching was affected bilaterally, although more severely in the contralateral forepaw and increased with lesion size. Rats with neonatal lesions showed significantly better reaching with the contralateral paw than the adults.

*After Whishaw and Kolb (1988).*
UNILATERAL VERSUS BILATERAL INJURY

An obvious difference between the unilateral and bilateral injuries is that in the former case there is an intact region homologous to the injured region, whereas in the bilateral case there is not. Two predictions from this difference are that we would expect better recovery from unilateral injury and the postinjury sequelae of brain changes are likely different. Both predictions are borne out.

Hicks and D’Amato (1975) were the first to show that when infant rats sustained unilateral lesions of the corticospinal projection neurons, they were subsequently found to have an anomalous ipsilateral pathway from the intact hemisphere to the spinal cord. They presumed that this pathway was responsible for the improved motor outcomes of the infants relative to adults with similar injuries. One prediction from their study is that rats with bilateral lesions in infancy (P1) should not show recovery because they would not have a normal hemisphere to project to the spinal cord. This is the case (Kolb et al., 2000a,b). Furthermore, in contrast to unilateral operates, the bilateral operates are impaired at tests of spatial navigation, in contrast to rats with adult lesions. This spatial deficit may be related to anomalous connections from the posterior cortical regions to the spinal cord, connections that likely were present at birth and failed to die after the early motor cortex lesions (e.g., O’Leary, 1989).

Although unilateral focal lesions provide an advantage in recovery, this advantage is reduced as the size of the lesion increases (Whishaw and Kolb, 1988). Furthermore, the larger the lesion, the greater is the impairment not only of the contralateral limb but also of the ipsilateral limb (Fig. 6).

Table 4 Summary of the effects treatments on recovery from early brain injury

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Outcome</th>
<th>Basic reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavioral therapy</td>
<td>Improved behavior</td>
<td>Kolb and Elliott (1987)</td>
</tr>
<tr>
<td>Complex housing</td>
<td>Improved behavior</td>
<td>Kolb and Gibb (2010)</td>
</tr>
<tr>
<td>Tactile stimulation</td>
<td>Improved behavior</td>
<td></td>
</tr>
<tr>
<td>Chemical therapy</td>
<td>Improved behavior</td>
<td></td>
</tr>
<tr>
<td>FGF-2 (P3 mPFC)</td>
<td>Improved behavior</td>
<td>Comeau et al. (2007a,b)</td>
</tr>
<tr>
<td>FGF-2 (P10 Motor)</td>
<td>Nearly normal behavior</td>
<td>Monfils et al. (2006)</td>
</tr>
<tr>
<td>Manipulation of gonadal hormones</td>
<td>Impaired recovery</td>
<td>Kolb and Stewart (1995)</td>
</tr>
<tr>
<td>Prenatal experiences</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGF-2</td>
<td>Improved behavior</td>
<td>Comeau et al. (2007a,b)</td>
</tr>
<tr>
<td>Tactile stimulation</td>
<td>Improved behavior</td>
<td>Gibb (2004)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Improved behavior</td>
<td>Kolb et al. (2008)</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Blocked recovery</td>
<td>kolb et al. (2008)</td>
</tr>
<tr>
<td>Excessive exercise</td>
<td>Reduced recovery</td>
<td>Gibb (2004)</td>
</tr>
<tr>
<td>Gestational stress</td>
<td>Blocked recovery</td>
<td>Halliwell (2011)</td>
</tr>
</tbody>
</table>
The extreme form of a unilateral lesion is hemidecortication or hemispherectomy. As shown in Fig. 3, hemidecorticates are severely impaired with both limbs, but the neonatal (P2) hemidecorticates are significantly better with their contralateral limb than the adult decorticates. This is correlated both with considerable rewiring of the normal hemisphere and also with hypertrophy of pyramidal neurons throughout the intact hemisphere (Kolb et al., 1992). Curiously, whereas rats with bilateral focal lesions on P10 are always functionally advantaged relative to those with lesions at P1–6, this is not true with hemidecortication (Kolb and Tomie, 1988). In contrast, the early operates fare better and have more extensive anatomical remodeling. The difference between age-related differences between focal lesions and hemidecortication begs for more study.

7 FACTORS INFLUENCING BRAIN DEVELOPMENT IN THE INJURED BRAIN

The obvious place to look for beneficial modulating effects on the early-injured brain is at the animals with the earliest focal lesions. After all, these are the animals with the worst functional outcomes in the absence of treatment. Because most of the studies on modulating factors have been using rats with medial frontal lesions, our discussion will focus on this preparation, with reference to other lesions where appropriate. We have grouped the factors into (1) behavioral therapy, (2) pharmacological therapy, and (3) prenatal therapy (see Table 4). We discuss each in turn.

7.1 Behavioral therapy

Although behavioral therapy would seem to be the most obvious type of treatment to study, there are surprisingly few studies in either laboratory animals or human children and virtually no evidence regarding when the optimal time might be or how intense it ought to be. We began our studies by looking at complex housing. Placing animals with bilateral P1–7 mPFC lesions in complex housing for 90 days beginning at weaning stimulates significant functional improvement on tests of both cognitive and motor behaviors and this is correlated with increased cortical thickness, brain weight, and dendritic length (Kolb and Elliott, 1987). In contrast, placing the animals in the complex environments as adults was ineffective (Comeau et al., 2008), unless the lesions are unilateral (Williams et al., 2006).

Tactile stimulation is also an effective treatment. Rats were given mPFC on P3 and then beginning on P4 they received two weeks of tactile stimulation for 15 min 3× per day (Kolb and Gibb, 2010). The improvement on both spatial learning and skilled motor function was remarkable (Fig. 4) and was correlated with increased spine density in the tactile-stimulated mPFC rats. One possible mechanism for the effect of tactile stimulation is that the tactile stimulation increases the expression of FGF-2 in both skin and brain providing a possible mechanism for the therapeutic benefits.
abilities of tactile stimulation (Gibb, 2004). We note parenthetically that complex housing also increases FGF-2 expression in cortex (Kolb et al., 1998).

### 7.2 Chemical therapy

In view of the apparent role of FGF-2 in recovery, we subcutaneously administered FGF-2 directly as a therapy after P4 mPFC lesions and found significant functional improvement (Comeau et al., 2007a,b, 2008). In parallel studies, we administered FGF-2 to rats with P3 or P10 motor cortex lesions. The FGF-2 essentially reversed all of the motor deficits in the P10, but not the P3 lesion animals, but more importantly, the lost motor cortex tissue regenerated and formed corticospinal connections (Monfils et al., 2006, 2008) (Fig. 7). Pretreating the rats with BrdU on E12 blocked the FGF-2-mediated regeneration and prevented functional recovery, just as it did after the spontaneous regeneration after P10 mPFC lesions (see above).

Not all chemical treatments are beneficial, however. Perinatal depletion of noradrenaline on P1–3, administration of testosterone to female rats on P1, or blockade of gonadal hormones by gonadectomy on P1 both block recovery and the associated dendritic changes following P7 mPFC lesions (Kolb and Stewart, 1995; Kolb and Sutherland, 1992). Furthermore, even FGF-2 can be detrimental. In one study

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**FIGURE 7**

Sagittal brain sections illustrating normal motor cortex (A), a P10 motor cortex lesion (B), and a P10 motor cortex lesion and FGF-2 treatment (C). Compared to control cortex, the FGF-2 stimulated regrowth does not illustrate a laminar distribution. Scale bars in (A), (B), and (C) are 550 μm. Scale bars in (D) and (E) are 250 μm.

After Monfils et al. (2008).
performed in our laboratory, we gave FGF-2 and placed animals in complex housing at weaning (Hastings, 2003). This combination made the animals worse, possibly because the combined treatments raised FGF-2 to a toxic level.

### 7.3 Prenatal therapy

Given that prenatal events such as gestational stress alter brain development, we wondered if prenatal events might influence recovery from perinatal injury. They do. Prenatal tactile stimulation (i.e., of the pregnant mom), prenatal diazepam, and prenatal FGF-2 all enhance recovery from P3 mPFC lesions (Comeau et al., 2008; Gibb, 2004; Kolb et al., 2008). In contrast, prenatal administration of fluoxetine, prenatal stress, or excessive maternal exercise block recovery of rats with P7 mPFC lesions (Day et al., 2003; Gibb, 2004; Halliwell, 2011). The powerful effects of prenatal events, both positive and negative, demand further study as they likely can account for some of the variance in outcomes observed in children with perinatal perturbations such as ischemia.

### 8 SUMMARY

We have shown that the developing normal and injured brain shows a remarkable capacity for plastic change, both for better and worse. A key remaining question is how this happens. Although we have not discussed it here one consistent observation in both the normal and injured brain is a sex difference in experience-dependent plasticity. It is not simply that one sex is more plastic but rather that there is a sexual dimorphism in both the functional and anatomical effects of experiences. One clear example is the effect of prenatal stress on gene expression (Mychasiuk et al., 2011b). Although there are large changes in gene expression in the prefrontal cortex of each sex, there are few overlapping gene expression changes. But both sexes show a marked alteration in dendritic organization, suggesting that there are multiple mechanisms underlying the observed plastic changes. This is clearly grist for future studies.

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